Clin Chem Lab Med 2009;47(9):1047–1050 © 2009 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2009.242

Peroxisome proliferator-activated receptor-γ (*PPARγ*) Pro12Ala polymorphism and risk for pediatric obesity

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Abstract

Background: Variation in the peroxisome-proliferator-activated receptor γ (*PPARγ*) gene has been reported to alter the risk for adiposity in adults.
Methods: We investigated the gender related association between the Pro12Ala variant (rs1801282) in obesity and insulin resistance traits in 794 peri-adolescent children aged 10–12 years of Greek origin from the Gene and Diet Attica Investigation (GENDAI) cohort.
Results: Gender stratified analysis suggested that in peri-adolescent boys, Ala carriers exhibited lower measures of skinfold (triceps: 16.9±6.9 vs. 19.4±7.9 mm, p=0.014; subscapular: 9.6±4.5 vs. 11.2±5.4 mm, p=0.016) and lower adiponectin concentrations (3.9±1.3 vs. 4.7±2.4 μg/mL, p=0.05). In peri-adolescent girls, Ala carriers had lower insulin concentrations (7.3±3.7 vs. 8.5±4.4 μU/mL, p=0.026) and lower values of homeostasis model assessment of insulin resistance (HOMA-IR) (1.5±0.8 vs. 1.8±0.96, p=0.019). Linear regression analysis revealed that the presence of the Ala allele in boys was a nominally significant predictor of obesity indices, including skinfolds (triceps: β±SE: –2.3±1.1, p=0.032; subscapular: β±SE: –2.3±1.1, p=0.04) and adiponectin concentrations (β±SE: –0.7±0.4, p=0.05) after adjusting for potential covariates. In girls, the Ala allele was a predictor of insulin concentrations (β±SE: –1.2±0.6, p=0.037) and HOMA-IR (β±SE: –0.24±0.13, p=0.037).
Conclusions: Our results suggest that adiposity in children is influenced by the Pro12Ala polymorphism in a gender specific manner.
Clin Chem Lab Med 2009;47:1047–50.

Keywords: adiposity; children; diet; gender interaction; obesity; peroxisome proliferator-activated receptor γ (PPARγ); Pro12Ala polymorphism.

Introduction

Childhood obesity is considered to be a rapidly growing health problem worldwide (1). It affects numerous body systems. Most notably, increased weight has been related to hypertension, dyslipidemia, increased tendency for blood clotting, and insulin resistance. The factors that regulate body fat content and distribution are not fully understood. With regard to dietary intake, there is no scientific consensus whether macronutrient composition affects energy metabolism and body fat distribution, beyond energy content. However, specific macronutrient composition of the diet may be one of the important environmental factors that control nutrient partitioning to specific adipose tissue depots, without affecting total body weight (2).

The human peroxisome proliferator-activated receptor-γ (PPARγ) plays a key role in the regulation of lipid and glucose homeostasis, in the differentiation of adipocytes and in fatty acid storage (3, 4). The Pro12Ala polymorphism has been widely investigated in relation to various disorders including obesity (5–7). However, the association of the Ala allele with a higher risk of obesity has not been consistent in all studies, suggesting heterogeneity in its effect. Alternatively, a combination of chance findings and low power may also contribute to differing results. It has been shown that *PPARγ* expression is attenuated in visceral adipose tissue in lean subjects (8, 9). This may suggest a link between the Pro12Ala polymorphism and regulation of regional adiposity and body weight.

In light of the increasing prevalence of pediatric obesity, it is important to assess the potential impact of the Pro12Ala polymorphism in the *PPARγ* gene at an earlier stage of life. This has been attempted by several groups, with largely inconsistent findings (10–13). It has recently been shown that in young children (13), the Pro12Ala polymorphism is associated with increased adiposity exclusively in girls. The mismatch regarding findings from adult and pediatric cohorts are likely due to the influence of unmeasured factors, such as diet, or age-specific effects (14). In the present study, a gender related association between

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Received April 29, 2009; accepted June 16, 2009; previously published online July 27, 2009

the Pro12Ala variant of the *PPARγ* gene and obesity traits in children was investigated.

Subjects and methods

Subjects

The Gene and Diet Attica Investigation (GENDAI) target population was children attending the fifth and sixth grades living in the Attica region of Greece, as described previously (15). The GENDAI cohort is comprised of 1138 adolescent children (53% girls; mean age: 11.2±0.7 years) randomly selected from elementary schools in Attica. The research study was approved by the Institutional Review Board of Harokopio University, and the Greek Ministry of Education. Informed consent for genetic analysis of all participants was obtained from the children's parents.

Dietary methodology

Dietary information regarding GENDAI study was evaluated through two non-consecutive 24 h recalls, three to ten days apart. The 24 h recalls were analyzed using the Nutritionist Pro software, version 2.2 (Axxya Systems-Nutritionist Pro Inc, Stafford, TX, USA). The mean nutrient intake from the two recalls was used for estimation of usual nutrient intake.

Physical activity assessment

Coinciding with the dates of the dietary recalls, participants completed a physical activity checklist recall two times (16). This instrument queried the child's time spent on mild, moderate, and strenuous exercise, plus sedentary pursuits, such as time spent viewing TV or playing computer/video games, during the previous 24 h. Then, the mean MET (metabolic cost of activity) scores were calculated from the children's reported history of physical activity, taking into account minutes of activity as well as minutes of inactivity.

Sexual maturity assessment

Sexual maturity status, according to Tanner's criteria for breast, pubic hair, and genital development (17) was self-evaluated. In particular, girls rated breast and pubic hair development and boys rated genital development and pubic hair development using a series of standardized photos, in the presence of the team's pediatrician.

Anthropometry assessment

Physical measurements of body weight and height were obtained in light clothing without shoes. Body mass index (BMI) was computed as weight (kg)/height (m^2). In addition, a soft tape measure was used to record waist circumference (cm) and hip circumference (cm). The waist-to-hip ratio (WHR) was calculated as an index of central adiposity. Two measurements of right side triceps and subscapular skinfolds were performed with Lange skinfold callipers (Cambridge Scientific Instruments, Cambridge, MA, USA) to obtain a mean measurement with precision of 0.2 mm.

Blood sampling

Ten mL of venous blood was collected after an overnight fast (≥10 h). The samples were placed in plain tubes as well as EDTA-containing tubes to obtain separate aliquots for serum and plasma. We present data on 794 children (420 girls and 374 boys) for which complete information and high-quality genotype data was available.

Genotyping

Genotyping of the Pro12Ala polymorphism (rs1801282) was done as part of a larger panel and was performed using the iPLEX MassARRAY platform (Sequenom). (http://www.sequenom.com/Assets/pdfs/appnotes/8876-006.pdf).

Statistical methods

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The normal distribution of the investigated variables was assessed using the Kolmogorov-Smirnov criteria. For all analyses we used log-transformed values for measures that were not normally distributed, namely BMI and waist. However, in our Tables, untransformed mean values are presented. Categorical data are presented as frequencies or proportions (%). Distributions of frequencies of categorical variables were analyzed using the χ^2-test of independence. The association of genotypes with adiposity outcomes was tested using multiple linear regressions, after controlling for the effects of gender and minutes of sedentary activity, as these variables were significant predictors. Interaction terms between genotypes and gender were also tested in each model and when significant, the analysis was stratified by gender. The critical p-value at which significance was accepted, was an α value=0.05. All reported p-values are based on two-sided tests.

Results

The overall frequency of the Ala allele was f(Ala:0.8), and the genotype frequencies were in Hardy-Weinberg equilibrium. Due to the small number of Ala12Ala homozygotes (n=6), all analyses were performed under a dominant genetic model. Gender stratified analysis suggested that in peri-adolescent boys, Ala carriers exhibited lower measures of skinfolds (triceps: 16.9±6.9 vs. 19.4±7.9 mm, p=0.014; subscapular: 9.6±4.5 vs. 11.2±5.4 mm, p=0.016) and lower adiponectin concentrations (3.9±1.3 vs. 4.7±2.4 μg/mL, p=0.05) (Table 1). No significant difference was observed between genotype groups for age, Tanner stage, sedentary activity, energy and different types of fat intake (expressed as % of total energy), or for the other biochemical markers.

In peri-adolescent girls, Ala carriers had lower concentrations levels (7.3±3.7 vs. 8.5±4.4 μU/mL, p=0.026), homeostasis model assessment of insulin resistance (HOMA-IR) (1.5±0.8 vs. 1.8±0.96, p=0.019) and saturated fatty acids (SFA) intake (14.8±3 vs. 13.5±2.9%, p=0.002) compared with the Pro/Pro girls (Table 1). These results persisted after adjustment for BMI for insulin concentrations (β±SE: −1.2±0.6, p=0.037), HOMA-IR (β±SE: −0.24±0.13, p=0.037) and SFA intake (β±SE: −1.2±0.4, p=0.003) (Table 2). In summary, linear regression analysis after adjusting for age, Tanner stage and minutes of inactivity, revealed that the presence of the Ala allele in boys was a nominally significant predictor of obesity indices, such as skinfolds (triceps: β±SE: −2.3±1.1, p=0.032; subscapular: β±SE: −2.3±1.1, p=0.04), and

Table 1 Anthropometric, pubertal stage, dietary intake, adiposity and biochemical parameters depending on the Pro12Ala polymorphism.

	Girls			Boys		
	Pro/Pro (n=356)	Ala allele (n=64)	p-Value	Pro/Pro (n=313)	Ala allele (n=61)	p-Value
Age, years	11.1±0.6	11.2±0.7	0.43	11.2±0.7	11.1±0.6	0.36
Tanner stage, % (I/II/III/IV/V)	9.3/33.7/39.9/14.6/2	14.1/26.6/50/9.4	0.35	12.5/45.4/31/8.9/1.9	14.8/44.3/34.4/6.6	0.44
Sedentary activity, min/day	113.9±74.6	114.9±83.9	0.92	143±102	127±77	0.25
Energy, kcal/day	1764±524	1788±543	0.73	2008±596	2024±751	0.87
TF, % of total energy	40±6.5	38.5±5.8	0.052	40.2±7	41±7	0.41
SFA, % of total energy	14.8±3	13.5±2.9	**0.002**	14.7±3.6	15±3.7	0.52
MUFA, % of total energy	16.2±4.3	16.2±4.2	0.99	16.4±4.2	16.8±4.4	0.53
PUFA, % of total energy	4.7±1.5	4.7±1.6	0.73	4.9±1.5	4.6±1.4	0.23
Anthropometry						
Weight, kg	44.2±9.4	43.6±10.3	0.57	44.8±9.4	42.9±9.3	0.13
BMI, kg/m^2	19.9±3.4	19.6±3.6	0.55	20.4±3.4	19.7±3.4	0.11
Skinfolds						
Triceps, mm	19.8±7.2	20±8.0	0.92	19.4±7.9	16.9±6.9	**0.014**
Subscapular, mm	11.7±5.3	12.3± 6.1	0.94	11.2±5.4	9.6±4.5	**0.016**
Waist circumference, cm	67.3±9.1	68.9±9.6	0.67	70.7±9.6	68.7±9.5	0.10
Body fatness, Slaughter equation	31.4±11.5	31.9±12.5	0.80	26.2±10.7	22.9±7.9	0.063
Biochemical indices						
Total cholesterol, mg/dL	184.3±28.3	181.2±28.4	0.49	190±26.9	186.7±27.2	0.38
HDL-cholesterol, mg/dL	51.4±9.7	51.2±10.9	0.90	53.4±10.1	53.4±9.9	70.99
LDL-cholesterol, mg/dL	119.2±23	118.3±21.9	0.76	124±21.9	120.1±23.2	0.23
Triglycerides, mg/dL	66.7±22	63.4±20.4	0.30	60.3±20.9	66.1±26.5	0.12
Glucose, mg/dL	85.1±8.6	84.1±8.5	0.40	86.8±8.4	85.6±8.9	0.31
Insulin, mg/dL	8.5±4.4	7.3±3.7	**0.026**	7.2±3.9	6.5±3	0.24
HOMA-IR, %	1.8±0.96	1.5±0.8	**0.019**	1.6±0.94	1.6±1	0.96
Leptin, pg/mL	7247±6471	7150±7174	0.94	5973±451	7212±1275	0.42
Adiponectin, μg/mL	4.5±2.2	4.7±2.7	0.42	4.7±2.4	3.9±1.3	**0.032**

Data are presented as means±SD. Values in bold font type. TF, total fat; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; BMI, body mass index; HOMO-IR, homeostasis model assessment of insulin resistance. Bold: $p<0.05$. To transform to SI units, the conversion factors are: total cholesterol, HDL-, LDL-cholesterol×0.026; glucose×0.055; triglycerides×0.011; insulin×0.0417.

adiponectin concentrations (β±SE: –0.7±0.4, p=0.05) (Table 2).

Discussion

Our study extends previous findings (13) in children and suggests that there is a gender specific association of the Pro12Ala polymorphism with measures of adiposity at certain ages. While the frequency of the Ala12 allele (0.08) was similar to that reported in two Greek cohorts of children at different ages (0.07) (13, 18), no significant associations between *PPARγ* variants and anthropometric indices were identified in our pediatric cohort before gender stratification. Peri-adolescent boys with the Ala allele had lower skinfold measures (triceps and subscapular) compared to those with the Pro allele. This novel effect in children seems consistent with reports suggesting a protective effect of the Ala allele against obesity and improved insulin sensitivity in adults (19). Similar findings on obesity indices, depending on gender, were shown previously in adults (20) as well as children (21). These gender differences might be attributed either to sex-linked genes and/or variations in sex hormones. The different fat distribution patterns seen in the two genders could partially explain the etiology of sex variability on anthropometry, depending on the genotype. The lower concentrations of fasting blood insulin and lower HOMA-IR values that we found in girls confirm previous findings in obese Italian children (11). Additionally, female Ala carriers had a sig-

Table 2 Linear regression analysis stratified by gender for adiposity measures and insulin resistance.

Boys			Girls		
Dependent variables	β±SE	p-Value	Dependent variables	β±SE	p-Value
Triceps, mm	2.3±1.1	**0.032**	Insulin, mg/dL	1.2±0.6	**0.037**
Subscapular, mm	2.3±1.1	**0.04**	HOMA-IR	0.24±0.13	**0.037**
Adiponectin, μg/mL	0.7±0.4	**0.05**	SFA, % of total energy	1.2±0.4	**0.003**

Adjustment has been made for age, Tanner stage and sedentary activity for all variables. Adiponectin and saturated fat (% of total energy) were additionally adjusted for BMI. B-coefficients are values comparing Pro/Pro with Ala carriers. HOMO-IR, homeostasis model assessment of insulin resistance; SFA, saturated fatty acids; SE, standard error. Bold: $p<0.05$.

nificantly lower intake of SFA. It was previously shown in type 2 diabetic patients that carriers of the Ala allele had a lower energy intake per kilogram of body weight, suggesting the Ala allele was associated with greater food efficiency (7). The lower concentrations of adiponectin found in boys with the Ala allele were also seen in young adult males from a Japanese cohort (22), suggesting interaction with the adiponectin gene. The screening analysis using BMI did not reveal any significant association with the Ala allele, which is consistent with recent results from an 11 to 24-year-old cohort from Mexico (1210 students) (12).

In summary, we found evidence of a possible effect of the Ala allele at the Pro12Ala locus in the *PPARγ* gene on adiposity indices in boys, and SFA intake and insulin indices in girls. These results add to the epidemiologic data for this variant in children. The modest study size or heterogeneity of the effect in boys and girls at this age may account for the gender specific findings.

The cross-sectional design does limit the potential to reveal causal relationships. However, these findings may motivate further study in larger cohorts with longitudinal data in order to more fully understand the impact of this gene variant on growth and development of obesity and diabetes in boys and girls.

Acknowledgements

The authors would like to thank the field investigators for their assistance in physical examination, biochemical analysis and nutritional assessment. Above all, the authors thank the study participants and their families for their time and effort to help create both paediatric cohorts. The collection of the GENDAI cohort was funded by Coca-Cola Hellas. The genotyping was funded by NIDDK K23DK067288 awarded to HNL. All authors were responsible for reviewing the manuscript. We declare that we have no conflict of interests.

References

1. Deckelbaum RJ, Williams CL. Childhood obesity: the health issue. Obes Res 2001;9(Suppl 4):239S–43S.
2. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs. saturated fat (cream). Int J Obes Relat Metab Disord 2002;26:814–21.
3. Argmann CA, Cock TA, Auwerx J. Peroxisome proliferator-activated receptor gamma: the more the merrier? Eur J Clin Invest 2005;35:82–92; discussion 80.
4. Heikkinen S, Auwerx J, Argmann CA. PPARgamma in human and mouse physiology. Biochim Biophys Acta 2007;1771:999–1013.
5. Barbieri M, Rizzo MR, Papa M, Acampora R, De Angelis L, Olivieri F, et al. Role of interaction between variants in the PPARG and interleukin-6 genes on obesity related metabolic risk factors. Exp Gerontol 2005;40:599–604.
6. Danawati CW, Nagata M, Moriyama H, Hara K, Yasuda H, Nakayama M, et al. A possible association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma2 gene with obesity in native Japanese in Indonesia. Diabetes Metab Res Rev 2005;21:465–9.
7. Vaccaro O, Lapice E, Monticelli A, Giacchetti M, Castaldo I, Galasso R, et al. Pro12Ala polymorphism of the PPARgamma2 locus modulates the relationship between energy intake and body weight in type 2 diabetic patients. Diabetes Care 2007;30:1156–61.
8. Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, et al. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 1998;47:98–103.
9. Memisoglu A, Hu FB, Hankinson SE, Manson JE, De Vivo I, Willett WC, et al. Interaction between a peroxisome proliferator-activated receptor gamma gene polymorphism and dietary fat intake in relation to body mass. Hum Mol Genet 2003;12:2923–9.
10. Cecil JE, Fischer B, Doney AS, Hetherington M, Watt P, Wrieden W, et al. The Pro12Ala and C-681G variants of the PPARG locus are associated with opposing growth phenotypes in young schoolchildren. Diabetologia 2005; 48:1496–502.
11. Scaglioni S, Verduci E, Salvioni M, Biondi ML, Radaelli G, Agostoni C, et al. PPAR-gamma2 Pro12Ala variant, insulin resistance and plasma long-chain polyunsaturated fatty acids in childhood obesity. Pediatr Res 2006;60: 485–9.
12. Chen L, Velasco Mondragon HE, Lazcano-Ponce E, Collins A, Shugart YY. Effect of the peroxisome proliferators-activated receptor (PPAR) gamma 3 gene on BMI in 1,210 school students from Morelos, Mexico. Pac Symp Biocomput 2006:467–77.
13. Lagou V, Scott RA, Manios Y, Chen TL, Wang G, Grammatikaki E, et al. Impact of peroxisome proliferator-activated receptors gamma and delta on adiposity in toddlers and preschoolers in the GENESIS Study. Obesity (Silver Spring) 2008;16:913–8.
14. Lasky-Su J, Lyon HN, Emilsson V, Heid IM, Molony C, Raby BA, et al. On the replication of genetic associations: timing can be everything! Am J Hum Genet 2008;82: 849–58.
15. Papoutsakis C, Vidra NV, Hatzopoulou I, Tzirkalli M, Farmaki AE, Evagelidaki E, et al. The Gene-Diet Attica investigation on childhood obesity (GENDAI): overview of the study design. Clin Chem Lab Med 2007;45:309–15.
16. Sallis JF, Strikmiller PK, Harsha DW, Feldman HA, Ehlinger S, Stone EJ, et al. Validation of interviewer- and self-administered physical activity checklists for fifth grade students. Med Sci Sports Exerc 1996;28:840–51.
17. Tanner JM. The measurement of maturity. Trans Eur Orthod Soc 1975:45–60.
18. Dedoussis GV, Theodoraki EV, Manios Y, Yiannakouris N, Panagiotakos D, Papoutsakis C, et al. The Pro12Ala polymorphism in PPARgamma2 gene affects lipid parameters in Greek primary school children: a case of gene-to-gender interaction. Am J Med Sci 2007;333:10–5.
19. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284–7.
20. Morini E, Tassi V, Capponi D, Ludovico O, Dallapiccola B, Trischitta V, et al. Interaction between PPARgamma2 variants and gender on the modulation of body weight. Obesity (Silver Spring) 2008;16:1467–70.
21. Jacobsson JA, Danielsson P, Svensson V, Klovins J, Gyllensten U, Marcus C, et al. Major gender difference in association of FTO gene variant among severely obese children with obesity and obesity related phenotypes. Biochem Biophys Res Commun 2008;368:476–82.
22. Takata N, Awata T, Inukai K, Watanabe M, Ohkubo T, Kurihara S, et al. Pro12Ala substitution in peroxisome proliferator-activated receptor gamma 2 is associated with low adiponectin concentrations in young Japanese men. Metabolism 2004;53:1548–51.